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13. ABSTRACT *(Maximum 200 words)*

Aromatic acids are intermediates in the biodegradation of structurally diverse aromatic compounds, including lignin monomers and environmental pollutants, by many metabolic types of anaerobic bacteria. They are also the starting compounds for central pathways of anaerobic benzene ring reduction and fission. We have identified and developed molecular tools that can be used to manipulate and clone genes for aromatic acid degradation from the bacterium, Rhodopseudomonas palustris. These tools have enabled us to identify genes specifying two enzymes that initiate the degradation of the compounds benzoate and 4-hydroxybenzoate, and we have also cloned, sequenced, and characterized a regulatory gene required for the expression of aromatic acid degradation enzymes. Thus, the first steps towards elucidating the molecular basis for benzene ring fission in the absence of oxygen have been accomplished.

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MOLECULAR BIOLOGY OF ANAEROBIC AROMATIC BIODEGRADATION

FINAL REPORT

CAROLINE S. HARWOOD

AUGUST 14, 1992

U. S. ARMY RESEARCH OFFICE
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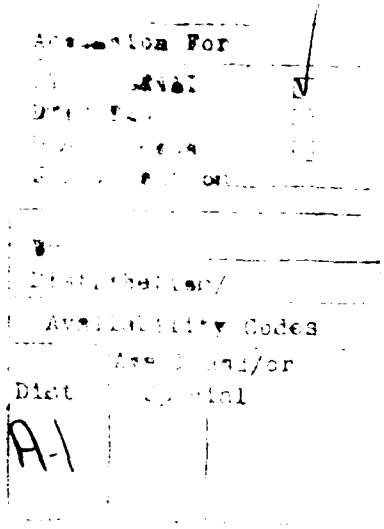

A. STATEMENT OF THE PROBLEM STUDIED.

Chlorinated aromatic compounds and aromatic hydrocarbons, including toluene and xylene, comprise a large proportion of the toxic wastes that have been released into the environment. Under anaerobic conditions the aromatic carboxylic acids, benzoate and 4-hydroxybenzoate, are formed as key intermediates during the biodegradation of aromatic pollutants. These acids then enter central pathways of anaerobic benzene ring reduction and fission, leading to complete mineralization.

Not a single catabolic pathway for the anaerobic degradation of any aromatic compound has yet been worked out in detail, and the molecular basis for aromatic compound degradation by bacteria is even less well explored. If the potential of bacteria to degrade benzene rings under anaerobic conditions is to be manipulated to realize their full detoxification potential, or to produce intermediary compounds that may have commercial value, it will be necessary to understand in detail the metabolic mechanisms involved, to know how the pathways are regulated, and to develop approaches for modifying the genes encoding key enzymes.

As an approach to achieving these goals we have been studying the anaerobic degradation of two selected aromatic acids - benzoate and 4-hydroxybenzoate - by one bacterial species - *Rhodopseudomonas palustris*. Our emphasis has been on developing tools to explore the genetic basis of aromatic acid degradation. Our expectation is that it will be possible to extend many of our conclusions to other bacteria and to related compounds.

B. SUMMARY OF THE MOST IMPORTANT RESULTS.

Our most important contribution during the last three years has been the identification and development of molecular tools that can be used to clone and manipulate genes in *R. palustris*. These tools have enabled us to identify a regulatory gene, termed *aadR* (for anaerobic aromatic degradation regulator) which is required for the expression of genes involved in anaerobic 4-hydroxybenzoate and benzoate degradation. We have also obtained partial clones of the genes for benzoate-CoA ligase and aromatic acid-CoA ligase II, enzymes that catalyze the first steps in the degradation of benzoate and 4-hydroxybenzoate, respectively. Finally, we have made extensive use of immunoblot assays to identify environmental factors that are required for the regulated expression of benzoate-CoA ligase. With this work we have accomplished the first steps in elucidating the molecular basis for benzene ring fission in the absence of oxygen.

C. PUBLICATIONS.

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D. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED.

Marilyn Dispensa (Technician)
Joseph Perrotta (Graduate Research Assistant)
Min-Kyung Kim (Graduate Research Assistant) - M. S. awarded

E. REPORT OF INVENTIONS:

None